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**FIRST CANADIAN RECORD OF THE BERMUDA GRASS STEM MAGGOT, *ATHERIGONA REVERSURA* (DIPTERA: MUSCIDAE)**

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**Scientific Note**

The large muscid genus *Atherigona* Rondani (Diptera) includes over 230 species, most of which are found in the tropical and subtropical regions of the Old World (Pont and Magpayo 1995). The group is divided into two subgenera: *Acritochaeta* Grimshaw, which contains mostly saprophagous taxa, and *Atherigona* Rondani, whose phytophagous larvae are shoot-flies that feed on a variety of wild and cultivated grasses (Poaceae) (Pont and Magpayo 1995).

Only two species of *Atherigona* are currently found in North America. The cosmopolitan *Atherigona orientalis* Schiner (Diptera: Muscidae) has long been known from the southern United States (Malloch 1921; Hockett 1936) and has not, so far, been recorded in Canada. *Atherigona reversura* Villeneuve (Diptera: Muscidae), a widespread species in the Oriental, Australasian and Palearctic regions (Pont and Magpayo 1995), is a much more recent introduction: it was documented from the United States less than a decade ago (Hudson 2010) and is recorded here for the first time from Canada. The species was found to rapidly infest and damage Bermuda grass, *Cynodon dactylon* (Linnaeus) Persoon, from hayfields, pastures and turf in the southern United States (Baxter *et al* 2014), a habit that has resulted in the recent attribution of the common name Bermuda grass stem maggot (BSM).

The BSM is a small yellowish fly measuring 3.0–3.5 mm with a remarkably angular dichoptic head in both sexes and long antennae (Fig. 1A). It can be separated from *A. orientalis* and other members of the subgenus *Acritochaeta* Grimshaw based on the presence of very short basal lateral setae on the scutellum (these are almost as long as subbasal lateral setae in *A. orientalis*), 2–3 rows of presutural acrostical setae (4–5 rows in *A. orientalis*), and a clubbed palpus in the male (Fig. 1A) (male palpus elongate in *A. orientalis*). The combination of yellow palps (Fig. 1A), a bicoloured frontal vitta (Fig. 1B), and a moderately ornamented fore tarsus in the male (Figs. 95–96 of Pont and Magpayo 1995) will distinguish *A. reversura* from other species belonging to the subgenus *Atherigona sensu stricto*. The immature stages have been extensively described by Grzywacz *et al.* (2013). In addition to Bermuda grass, *A. reversura* has been reared from a variety of host-plants (see Pont and Magpayo 1995 for complete list) including some cultivated in Canada such as maize, *Zea mays* Linnaeus, and, only recently, sweet sorghum, *Sorghum bicolor* (Linnaeus) Moench, (Thivierge *et al.* 2015).

*Published March 2016*

The presence of the BSM in Southern Ontario represents new Canadian records at both the generic and species levels. While this species was first noticed in the United States of America due to the damage it caused to Bermuda grass, the Canadian specimens were discovered through an ongoing project on the DNA barcoding of the Muscidae of Canada based on a large database of COI Muscidae sequences from all over Canada (Savage *et al.* 2015). Eight sequences from that data set belonged to the Barcode Index Number (Ratnasingham and Hebert 2013) AAN8579 and were assigned to the genus *Atherigona* by the automated identification engine of the Barcode of Life Data System (BOLD, Ratnasingham and Hebert 2007). Upon further examination and genitalic dissections of the barcoded specimens, the material was identified as *A. reversura* (2 males, 6 females). All Canadian records reported here (Table 1) are from Ontario, with the oldest dating back to 2010. Collection details and individual sequences for the specimens listed here can be retrieved from BOLD in the public dataset: *Atherigona* of Canada ([dx.doi.org/10.5883/DS-ATOC](https://dx.doi.org/10.5883/DS-ATOC)).

Additional North American specimens of *A. reversura* housed in the Biodiversity Institute of Ontario (Guelph, Ontario) were also seen in the course of this work, including material from Florida and California, as well as one male and two females collected in late September 2010 from Barnstable County, Massachusetts, representing the most northern record in the United States to date for the BSM. All Canadian specimens are adults collected by Malaise trap and therefore no information related to host-plant is currently available.

It is not possible at present to determine if the Canadian records reported here represent multiple punctual introductions of the BSM rather than the establishment of the species in Canada and no information is available on the cold-hardiness of the species.

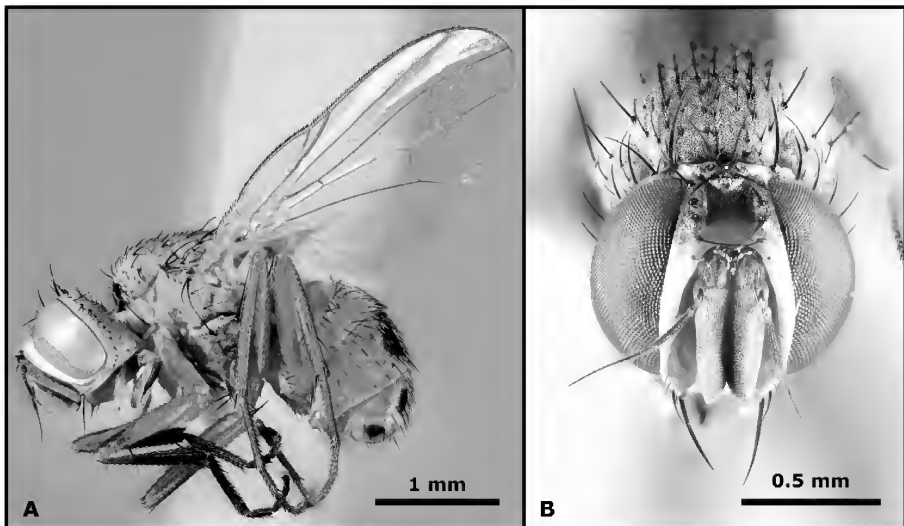


FIGURE 1: *Atherigona reversura*, male. A. habitus, lateral view; B. head, anterodorsal view.

Bermuda grass is considered an exotic weed in Canada, where it is found in Ontario and British Columbia (Plants of Canada Database 2011). However, since *A. reversura* is capable of completing its development in a wide range of other hosts, including some that are cultivated in Canada, the distribution of this species should be monitored in the future.

**Table 1.** Collection date, locality, number and sex of specimens and collection repository for specimens of *Atherigona reversura* from Canada; all localities are from Ontario. BIO = Biodiversity Institute of Ontario.

Date	Locality	Lat/Long	Specimen #	Collection repository
05.ix.2010	Leeds and Grenville, Elizabethtown-Kitley	44.621, -75.773	1♂	BIO, general collection
19.ix.2010	Haldimond-Dunn Townline, Windy Bluff	42.861, -79.703	1♂, 2♀	BIO, research collection of M. A. Smith
21.xi.2010	Haldimond-Dunn Townline, Windy Bluff	42.861, -79.703	1♀	BIO, research collection of M. A. Smith
12.ix.2012	Point Pelee National Park	41.939, -82.516	1♀	BIO, general collection
28.ix.2014	Walkerton, Sacred Heart High School	44.127, -81.144	1♀	BIO, general collection
28.ix.2014	London, Jack Chambers Public School	43.030, -81.271	1♀	BIO, general collection

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I particularly thank Paul Hebert and M. Alex Smith for access to the specimens and sequences, Jeremy deWaard for overseeing the collection program, and Valérie Lévesque-Beaudin for preparing the specimens and dataset. I also thank Michael Richardson and Véronique Bellavance from Bishop's University (BU) for providing digital images of specimens. Laboratory space was provided by BU and funding was provided in part by an NSERC Discovery Grant to J. Savage.

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## **A MODIFIED TECHNIQUE FOR REARING WOOD BORING INSECTS PERMITS VISUALIZATION OF LARVAL DEVELOPMENT**

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### **Abstract**

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Studying the larvae of wood boring insects is difficult because this stage of the life cycle is spent hidden from the eyes of researchers. Previous studies have used sections of bark and phloem sandwiched between pieces of glass or plastic to permit observation within the host substrate. The development of artificial diets for bark and wood boring insects has allowed the development of new techniques to study these insects under more consistent conditions. We modified an artificial diet in order to replicate the phloem sandwich technique by using a tortilla press to prepare sheets of artificial diet and then tested this technique by rearing larvae of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). We reared neonates and older larvae extracted from infested trees on the diet sandwiches and found rates of establishment and survivorship were higher for older larvae extracted from trees than for neonates. Our technique improves upon traditional phloem sandwiches by allowing all larvae to be reared under the same conditions and permitting the observation of larval behaviour even when the larva is submerged in the diet.

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### **Introduction**

Studying the ecology and behavior of bark and wood boring beetle larvae is difficult because this part of the life cycle is hidden from researchers. Bedard (1933) devised a solution to this problem by placing the inner bark of Douglas fir, *Pseudotsuga menziesi* (Mirb.) Franco (Pinaceae), between two glass slides held in place with elastic bands to study the Douglas-fir beetle *Dendroctonus pseudotsugae* (Hopkins) (Coleoptera: Curculionidae). Since then the technique has been modified, extended, and come to be known by the name ‘bark sandwiches’ or ‘phloem sandwiches’ (Yu and Tsao 1967; Beanlands 1966; Wermelinger and Seifert 1998).

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Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a highly destructive pest of ash, *Fraxinus* (Oleaceae). Studying the larval ecology of EAB has meant either removing larvae from infested ash (Wang *et al.* 2010), which kills the insect, or observing larvae in standing trees (Duan *et al.* 2010), which is logistically difficult. The recent development of an artificial EAB diet (Keena *et al.* 2015) has allowed new ways to study EAB under controlled conditions. We developed a “diet sandwich” method inspired by the phloem sandwich method using sheets of artificial diet sandwiched between two microscope slides. We then tested our ability to visualize EAB larvae in the diet as they developed and quantified survivorship and overall development time.

## Materials and Methods

All diet preparation was done under sterile conditions in the diet kitchen at Natural Resources Canada Canadian Forest Service, Great Lakes Forestry Centre (GLFC), Insect Production Unit, Sault Ste. Marie, Ontario, Canada. For these experiments we used the ingredient list from “diet #3” from Keena *et al.* (2015) (Table 1) with minor revisions. We used a cutter mixer (model HCM450, Hobart Canada, North York, Ontario) rather than a steam kettle to mix the ingredients, which resulted in more homogenous diet product. We then used a 23 cm diameter tortilla press (model Victoria-85008, Imusa USA, Doral, Florida, U.S.A) to make diet sheets and vacuum sealed the diet sheets for long-term storage.

TABLE 1. Ingredients of emerald ash borer artificial diet #3 from Keena *et al.* (2015).

Mix	Ingredient(s)	Quantity (g)
Starting base	Agar ( <i>Gracilaria</i> spp.)	220
	Sodium bicarbonate	5
Dry mix #1	Casein (edible grade)	200
	Sucrose	300
	Wesson salt mix without iron	45
	Sorbic acid	5
	Calcium propionate	5
	Methyl para benzene (Methyl paraben)	5
	Potato starch	200
Dry mix #2	Soy flour	500
Wet mix	Cholesterol	19
	Wheat germ oil	24
Vitamins and fibre	Choline chloride	4
	Vitamin A acetate beadlets	5
	Bioserv#F8128 (vitamin mix)	71
	Alphacel	1,500

**Preparation of diet and diet sandwiches**

1. Boil autoclaved reverse-osmosis water in a steam kettle and add sodium bicarbonate to neutralize the pH of the water.
2. Add agar with continuous stirring by spatula, so that no clumps form and heat until boiling.
3. Transfer the agar solution to a cutter mixer and add dry mix #1 (Table 1) and mix at 1140 rpm for 1 min.
4. Quickly add soy flour and mix at 1140 rpm for 1 min.
5. Add wet mix (Table 1) and mix at 1140 rpm for 3 min.
6. Allow the diet to cool until the temperature reaches 50C (this step is necessary as it prevents heat degrading the vitamins added in step 7).
7. Add dry mix #2 (Table 1) and mix at 1140 rpm for 1 min.
8. Add 750 g of Alphacel and mix it 1140 rpm for 1 min, then add remaining 750 g and mix again for 1 min.

The product from the above procedure should be the consistency of bread dough. To prepare the diet sheets we used the following procedure:

1. With gloved hands, break the large mass into 5 to 10 small balls, each approx. 10 cm in diameter.
2. Place the diet balls into closed plastic bags to prevent drying.
3. Spread 0.5 ml of wheat germ oil on a 60 × 30 cm piece of wax paper and place one end of the wax paper (oil side up) on a tortilla press.
4. Make small balls (4–4.5 cm diameter) from the large diet balls and place one on tortilla press on top of the wax paper.
5. Fold the remaining wax paper over the ball and flatten the press until the ball reaches the desired thickness (3–6 mm) (Fig. 1A).
6. Vacuum seal the diet sheets in plastic bags (10 sheets/bag) and store at 4C. The sheets are good for up to 6 months.

Finally, to prepare the diet sandwiches we used the following procedure:

1. Remove 1 diet sheet from the plastic bag and place one glass slide (7.5 × 5.1 × 0.1 cm; No. 2957, Erie Scientific Company, Portsmouth, New Hampshire) on top of the sheet.
2. With a scalpel, cut the diet sheet using the glass slide as a template.
3. Place the diet sheet between two glass slides to form a sandwich.
4. Wrap the sandwich in clear plastic wrap.
5. Prepare the desired number of sandwiches and store at 4C until needed.

**Introduction of larva on diet sandwich**

Larvae were introduced to the diet sandwiches by placing them into a small notch cut into the upper surface of the diet sandwich using a scalpel (Fig. 1B). Other methods suggest introducing EAB to the diet as eggs and allowing the neonates to hatch directly into the diet (Keena *et al.* 2015). However, we found that placing larvae allowed us to confirm that viable individuals are being used in our experiments. Individual diet sandwiches were then placed upright in small boxes (15 × 7 × 6.5 cm) inside an opaque plastic box (35 × 25

× 18 cm). A 1–2 cm deep layer of water was put in the opaque box to maintain humidity, and the small plastic boxes were placed on a plastic grid in the bottom of the opaque box to prevent them from being submerged. All larvae were reared in an environmental chamber (24C; 65% RH; 16L:8D). The developing larvae occasionally needed to be moved to a new diet sandwich (e.g., when the diet dried out, or when the larvae consumed a large portion of the diet) using the technique described above.

EAB larvae reared on an artificial diet typically stop feeding after the 4<sup>th</sup> instar and require a chill period to develop further (Keena *et al.* 2015). This part of the life cycle can be recognized by morphological changes in larval features such as the larvae reducing in length, becoming whiter and more opaque, the thoracic segments becoming narrower and shorter than the abdominal segments, and most notably, it stops feeding altogether and bends itself into a J-shaped configuration. The rearing temperature was gradually decreased to 16C for 7 d then to 10C for 7 d and finally to 4C for 84 d. After 84 d, larvae were removed from the diet sandwiches and transferred individually into wells of a 6-well culture plate

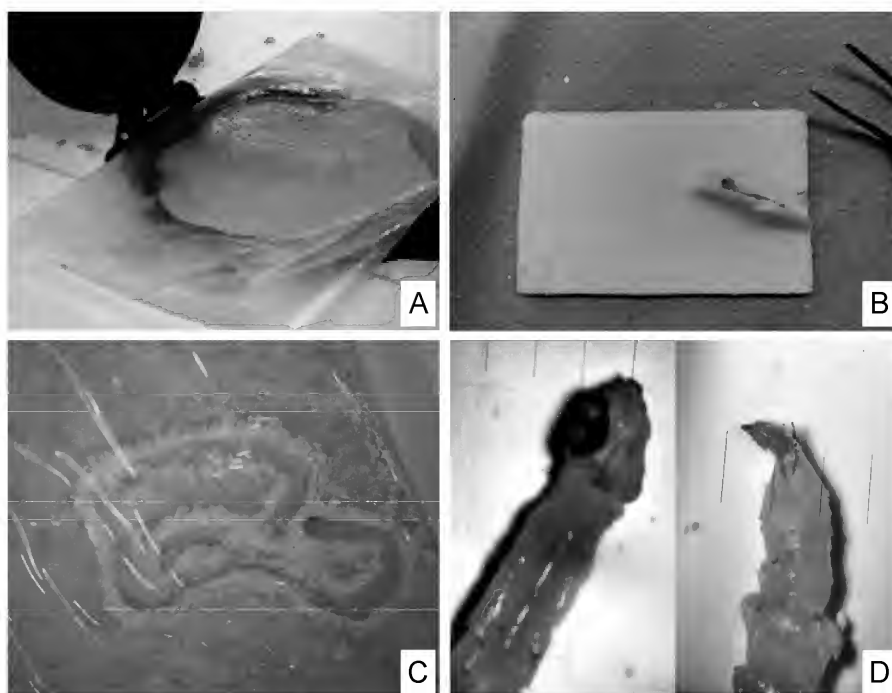


FIGURE 1: (A) Tortilla press used to press sheet of artificial emerald ash borer diet, (B) into which a larva is placed via a slit cut into the upper surface of the diet sandwich. (C) Implanted larvae create serpentine galleries in the artificial diet sandwich, (D) which can be used to follow the progress of development, including molting.

(Becton Dickinson Labware, Franklin Lakes, NJ, USA 07417-1886) for pupation and adult development. A moist cotton pad covered with 8 to 10 layers of paper towel was placed in the bottom of each well to provide a rough substrate to help with molting. Well plates were stored in the same opaque plastic boxes used to hold the diet sandwiches and maintained at the same rearing conditions (24C; 65% RH; 16L: 8D) until pupation. During this time the cotton pad and paper towels were re-moistened as necessary to maintain humidity and prevent fungal growth.

To demonstrate that diet sandwiches are a viable rearing technique, we evaluated the success of EAB development under the conditions described above. We tested the success of EAB larvae from two sources: those hatched from eggs (neonates, < 24 h old) and those extracted from infested ash trees (extracted larvae). We obtained EAB eggs from a colony maintained at GLFC which were allowed to hatch and neonate larvae were transferred to the diet sandwiches. Extracted larvae were either removed from sections of ash trees obtained from infested ash stands or removed from standing infested trees. Larvae were collected individually into well plates or petri plates with moistened filter paper, then immediately placed onto diet sandwiches. We placed 83 1<sup>st</sup>–4<sup>th</sup> instar extracted larvae, determined by measuring head capsule width (Chamorro *et al.* 2012) and 135 neonates on individual diet sandwiches. The moisture content of fresh diet and diet that had been stored for 6 months was determined using a moisture analyzer (Model MF-50, A&D Company Ltd, Toshima-ku, Tokyo, Japan).

## Results

EAB larvae began tunneling, and visible galleries could be seen 2 or 3 d after being placed on diet (Fig. 1C); however, some larvae took up to 30 d to begin feeding. Approximately half (52%) of the infested larvae established on the diet. We observed slightly higher establishment rates among extracted larvae (54%) than neonates (50%) (Table 2). However, 13.3% (n = 18) of the larvae infested as neonates escaped the diet and perished. Of the larvae infested as neonates, 6 reached the 4<sup>th</sup> instar, of which 4 were placed into the chill period but none converted into pupa. Twenty of the extracted larvae were placed into the chill period, of which 3 pupated and 2 emerged into adults. Comparing development times we found that, for larvae infested as neonates, development time to 3<sup>rd</sup> instar was (mean  $\pm$  1 s.d.) 73  $\pm$  40 d (n = 9) and to the 4<sup>th</sup> instar was 195  $\pm$  66 d (n = 6). For extracted larvae, development time to 3<sup>rd</sup> instar was 174  $\pm$  98 d (n = 9) and to fourth instar was 171  $\pm$  36 d (n = 8). For comparison, previous studies observed development times for male and female EAB of 176  $\pm$  11 d and 145  $\pm$  9 d, respectively, at 25  $\pm$  2C (Keena *et al.* 2015).

We found a small decrease in moisture in the diet during the 6 month storage period (59% for fresh diet vs. 54% for 6 month old diet).

## Discussion

We found that EAB larvae infested as neonates and extracted from ash adopted and fed on artificial diet sandwiches, and their development was visible through the glass slide. We also observed establishment (52%) on artificial diet sandwiches comparable to rates of

68% and 70% in earlier studies that used a version of the same artificial diet (Chen *et al.* 2011; Keena *et al.* 2015). The development times we observed were slightly longer than previous studies; however, these studies reared their insects at higher temperatures.

There are a number of factors that influence the success of insects feeding on an artificial diet. For instance, both high and low moisture content in artificial diets containing ash phloem increases the mortality of larvae (Chen *et al.* 2011). The optimal moisture content of EAB diet is 50 (Keena *et al.* 2015) or 60% (Chen *et al.* 2011), while the moisture content of our freshly made diet was 59% (54 after storage). By contrast, in living ash, the moisture content of phloem varies from 44% to 52% (Hill *et al.* 2012). This may in part explain the different performance of larvae in our experiment. In future it may be prudent to desiccate the diet before constructing sandwiches to more closely replicate moisture conditions in ash phloem. Introducing live larvae may also be too severe a trauma compared to introducing larvae from hatched eggs (Keena *et al.* 2015). Thus, while our method should result in a higher number of live larvae being placed on diet it may also reduce survivorship. We also lost a significant number of larvae due to them escaping the sandwich. This was not anticipated as larvae feeding in cut ash logs rarely escape the phloem, and we anticipated the same behavior would occur here. To resolve this, it may be necessary to use an impenetrable barrier around the edge of the diet sandwich to prevent the insect from escaping. Increasing the thickness of the diet sheet may also increase the success rate of EAB on diet entering the J-stage and successfully overwintering.

Diet sandwiches permit the observation of developing larvae in a consistent environment over time, without disturbing the larvae’s feeding process. Therefore we argue the method has use as a research tool for the study of larval wood borer behaviour and ecology (Fig. 1D). For instance, we have used the technique to determine developmental rates for EAB larvae (CJKM unpublished) and observed how galleries changed in size and conformation over time. We propose that the technique could be applied to the study of new insecticides or the role of phytochemicals in ash defensive response (Chakraborty *et*

TABLE 2. Percent establishment of emerald ash borer larvae from two sources implanted into diet sandwiches, and the final stage reached.

Source	Stage started	n	% established (n)	Number reaching stage (n)					
				L1	L2	L3	L4	Chill	Pupa
Reared	Neonates	135	49.6 (67)	26	26	9	6	4	0
Extracted	L1	19	57.9 (11)		4	3	2	1	1
	L2	27	62.9 (17)			2	5	9	1
	L3	24	37.5 (9)				5	4	0
	L4	13	61.5 (8)					7	1
	Total	83	54.2 (45)						

*al.* 2014). The advantage of diet sandwiches over traditional phloem sandwiches is that the method is not limited by the number of bark discs that can be collected, nor is it influenced by variability among trees that are used to obtain bark and phloem samples. Other studies have used crumbled artificial diet for bioassays in micro-centrifuge tubes to test for the effect of phytochemicals on larval development (Whitehill *et al.* 2014). However, that method does not simulate the feeding environment the insect normally experiences inside the tree, which could influence both the behaviour and subsequent survivorship of the insect. Using the diet sandwich technique we have also observed that even if the larva is immersed in the diet, it still can be observed by placing the sandwich in front of bright light. This permits continuous observation of larvae when they are submerged, a feature that is not usually possible with traditional bark and phloem sandwiches or crumbled diet in tubes. Moreover, we see no barrier to the technique being adapted to the study of other bark and wood boring beetles that can also be reared on artificial diets (Gould *et al.* 2004; Gindin *et al.* 2009).

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## EXCLUSION FENCING INHIBITS EARLY-SEASON BEETLE (COLEOPTERA) ACTIVITY-DENSITY IN BROCCOLI

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### Abstract

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Exclusion fencing represents a potentially useful management tool for key insect pests in broccoli but may also affect other invertebrates that have important roles in agroecosystems. Because beetles (Coleoptera) are generally abundant and diverse in agriculture and some species (i.e., members of the Carabidae and Staphylinidae) are important for biological control, pitfall traps were used in this study to compare beetle communities during late spring and early summer 2013 in fenced, unfenced and control plots of broccoli. Control plots were separated from fenced and unfenced plots to determine whether fencing increased captures in adjacent unfenced plots. Early on, fewer beetles (total and for most functional trait categories) were captured in fenced plots, but as the season progressed captures were similar among plot types. There was little evidence that fencing increased beetle diversity or activity density in adjacent unfenced plots, and later in the season some ground beetle species were instead strongly associated with control plots. Pitfall traps captured relatively high numbers of crucifer flea beetle, *Phyllotreta cruciferae* Goeze. Most captures were in unfenced and control plots early in the season, suggesting that fencing was effective in keeping this pest away from broccoli. Overall, fencing could limit or delay surface-active predatory beetles from accessing broccoli fields, having a negative effect on biological control services. However, since many beetles eventually permeated fencing, modifications to the fencing design may allow beetles entry, restrict exit, and allow increase of beetle communities to improve biological control services in fenced areas.

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## Introduction

Development of new methods for use in integrated pest management is needed in order to reduce reliance on insecticides and provide solutions to organic growers (Bomford *et al.* 2000a). Natural enemies may contribute to control, and cultural practices, such as crop rotation, field sanitation, removal of alternate host plants/weeds, trap cropping, and manipulation of planting dates, can be used to reduce pest populations (Acheampong and Stark 2004; Ito *et al.* 2005; Corlay *et al.* 2007; Hemachandra *et al.* 2007; Chen *et al.* 2009; Hummel *et al.* 2010; Jia-Ying *et al.* 2010). Exclusion fencing (i.e., panels of fine mesh surrounding a crop) has been used as a physical practice to reduce pest infestations in horticultural crops (Vernon and McGregor 1999; Bomford *et al.* 2000a, b; Wyss and Daniel 2004), but impacts on associated entomofauna have not been examined.

While exclusion fencing can be effective in reducing pest damage in cole crop production (Vernon and Mackenzie 1998), the purpose of this study was to assess fencing impacts on diversity and activity density of surface-dwelling beetles. Many of these beetles are generalist predators (Coleoptera: Carabidae, Staphylinidae) known to prey on important crop pests (Collins *et al.* 2002; Hajek *et al.* 2007; Renkema *et al.* 2014). However, within these two beetle families and others common in agriculture (eg., Coleoptera: Scarabaeidae, Curculionidae, Elateridae), many species may have other functional roles as detritivores, fungivores, phytophages, or rhizophages (Clough *et al.* 2007; Renkema *et al.* 2016). Thus, grouping beetles by feeding guild across taxa may provide a better prediction of expected ecosystem services (e.g., predation of crop pests, nutrient cycling) than the use of taxonomic groupings alone.

Exclusion fencing studies have shown that since many agricultural pests fly at their highest density ca. 30 cm above ground, fences taller than 30 cm are necessary to block pest movement into cropped areas (Tuttle *et al.* 1988; Vernon *et al.* 1989; Vernon and Mackenzie 1998; Vernon and McGregor 1999; Bomford *et al.* 2000a, b; Wyss and Daniel 2004). Fences create shelter and provide calm air, with the result that there may be 2 to 7 times more insects on the leeward compared to windward side (Pasek 1988). A windbreak effect of fencing increases the potential for high insect numbers along perimeters (Bomford *et al.* 2000b). Fencing and exclusion barriers have been most commonly used in conjunction with pitfall traps to reduce densities of surface-dwelling predators in experimental areas, resulting in greater pest populations (Chiverton 1987; Holland 1998; Menalled *et al.* 1999). In a few cases, creating “egress” boundaries to prevent generalist predator emigration reduced sentinel prey or pest population build-up compared to field areas without boundaries (Menalled *et al.* 1999; O’Neal *et al.* 2005).

Many surface-dwelling beetles, particularly ground beetles (Carabidae), use field margins and may immigrate to crop land during spring and summer (Woodcock *et al.* 2005; Werling and Gratton 2008). Therefore, beetle captures should be lower in fenced than unfenced plots, with the degree of difference dependent on how well fences exclude beetles and the proportion of field-resident beetles in total numbers captured. The distribution of beetles between fenced and unfenced plots may change as the season progresses and beetles find their way into fenced areas. Finally, since ground beetles aggregate at edges (Hansen

and New 2005), fencing may serve to increase beetle numbers in unfenced plots that are in close proximity to fenced plots compared to unfenced plots at a greater distance.

## Materials and Methods

### Study Site

This study was conducted in a broccoli, *Brassica oleracea* L. Italica group, field (24 × 30 m), at the University of Guelph Elora Research Station, established to examine effects of variety and fencing on swede midge, *Contarinia nasturti* (Kieffer), infestation (Evans and Hallett, unpublished data). In the fencing efficacy experiment, there were four treatment combinations (i.e., two broccoli varieties, ‘Bay Meadows’ and ‘Windsor’, either fenced or unfenced) replicated five times and arranged in a randomized complete block design, with replicates as blocks. The field was divided into twenty 3 × 5 m plots separated by a 2 m border, with each plot consisting of four rows of 12 broccoli plants, with 95 cm between row spacing and 45 cm between plant spacing. Broccoli seedlings were grown from seed (Stokes Seed Co., Thorold, Ontario, Canada) in a greenhouse at the University of Guelph, using 96-cell flat trays and potting media (Pro-mix: 75–85% sphagnum peat moss, coarse perlite, vermiculite; Premier Horticulture Ltd., Dorval, Quebec, Canada), and fertilized once per week (10-52-10 plant starter fertilizer, Plant Products Co. Ltd., Brampton, Ontario, Canada). Eight-week-old seedlings were transplanted into the field 7–9 May 2013. Plots were hand-weeded prior to the initial transplant of seedlings and again on 20 June 2013. No insecticides were applied during the experiment. In order to account for potential effects of fencing on neighbouring unfenced plots, eight control plots of the same dimensions were planted in a broccoli field adjacent to the fenced/unfenced plots with seedlings of the same age, using the same protocols.

One day after transplanting, four 1.5 m tall ‘no-see-um’ mesh panels (Telstar Window Service Ltd., Agassiz, BC, Canada) were arranged around each fenced plot in a 5.5 × 5.5 m square. The bottom edge of the mesh fencing was buried under ~10 cm of soil, such that no gap occurred between the fence and the soil surface. Fence panels had 25 cm mesh overhangs angled 45° outward and downward to prevent insects from flying over the top edge of the fencing (Pats and Vernon 1999; Vernon and McGregor 1999).

### Sampling Protocol

Epigeic beetles were sampled 17 June – 19 July 2013. One of the five blocks (two fenced and two unfenced plots) and two of the eight control plots were randomly selected for sampling each week. Each block and control plot was sampled once, so that every fenced and unfenced plot in the study site was sampled, and so that sampling results in the previous week(s) would not affect subsequent sampling results by trapping out resident beetles in each plot. The two control plots sampled in the first week were resampled in the fifth week. Plots were sampled for five consecutive days using pitfall traps constructed from clear plastic 10 cm diameter deli containers (500 ml, Solo Cup Company, Lake Forest, IL), filled one-quarter with a 50% propylene glycol solution, as described in Brunke *et al.* (2014). Pitfall traps were placed in pairs within the plots, with one trap placed randomly in an outer row of plants and a second trap placed randomly in an inner row. Traps were

dug into the soil so that the top edge was level with the soil surface, and were protected from rain with a plastic cover, supported by wire pegs and positioned 5–10 cm above the trap. Contact of trap edges to the soil surface was maintained every second day. Traps were removed after 5 days, and contents were washed with water through a 425  $\mu\text{m}$  sieve (Fisher Scientific, Ottawa, Ontario) and stored in 70% ethanol until further processing. All beetles were pinned, most were identified to genus or species with existing literature (Bousquet 2010; Brunke *et al.* 2012) and assistance from taxonomic experts (Steve Paiero, University of Guelph; Adam Brunke, Agriculture and Agri-Food Canada), and all are stored at the School of Environmental Sciences, University of Guelph.

### Data analysis

Beetle captures were summed over both pitfall traps per plot, and activity density and species richness were compared among plot types using individual-based rarefaction, extrapolating rarefaction curves to 500 individuals. To evaluate intraseasonal differences in beetle diversity, the first two sampling weeks were designated as the early period and the final three weeks as the late period. Rarefied species richness and Simpson's diversity index values ( $1/D$ ) were calculated per plot per period, extrapolating species richness estimates for each plot to the plot with the greatest captures overall. Beetle genera or species were assigned to a trophic group using available literature (Good and Giller 1991; Larochelle and Larivière 2003; Clough *et al.* 2007; Lundgren 2009; Brunke *et al.* 2012). Analysis of variance (ANOVA) was used to compare beetle activity density, rarefied extrapolated species richness, and diversity and activity density of each major functional group, among the three treatments. Sampling dates were included as random effects. Residuals were checked for normality of error variance, and activity density data for each functional group were square-root transformed. Back-transformed means and 95% confidence intervals are shown. EstimateS 9.1.0 was used to generate rarefaction estimates and Simpson's diversity index values (Colwell 2013), and JMP® software (SAS 2013) was used for ANOVA, with  $\alpha = 0.05$ .

Unconstrained ordination in CANOCO 5 (terBraak and Smilauer 2012) was used to examine differences in beetle community composition among plot types. Only species ( $\log [x + 1]$  transformed) with five or more total captures were used. Principle component analysis (PCA) was performed with data centered by species. A biplot with scaling of scores focused on inter-species correlations was generated with the 15 species having the largest relative weight in the analysis.

## Results

A total of 1,170 beetles were captured in this study, belonging to 11 families and 42 genera/species. A few beetles, 1.5% of the total, were identified only to family or subfamily (Table 1). About half as many beetles were captured in fenced compared to unfenced and control plots (Fig. 1), but species richness was not affected, as 95% CIs overlapped (data not shown). Lower captures in fenced plots were most apparent during the first two sampling weeks (Fig. 2). Indeed, beetle captures were greater in control than fenced plots during the

TABLE 1. Beetles captured in fenced, unfenced and control broccoli plots at Elora Research Station, June 17 – July 19, 2013. Percent calculated from total number of beetles captured.

Family	Genus/species	No. (%)	Functional Group
Anthicidae	<i>Anthicus</i> Paykull sp. 1	10 (0.8)	Scavenger
	<i>Anthicus</i> sp. 2	1 (0.1)	Scavenger
Carabidae	<i>Agonum muelleri</i> (Herbst)	2 (0.2)	Predator
	<i>Amara aenea</i> (DeGeer)	4 (0.3)	Omnivore
	<i>Amara obesa</i> (Say)	3 (0.3)	Omnivore
	<i>Amara</i> Bonelli sp.	1 (0.1)	Omnivore
	<i>Anisodactylus sanctaecrucis</i> (F.)	65 (5.6)	Omnivore
	<i>Bembidion quadrimaculatum</i> Say	34 (2.9)	Predator
	<i>Bembidion</i> Latreille sp.	4 (0.3)	Predator
	<i>Bradycellus</i> Erichson sp.	1 (0.1)	Predator
	<i>Clivina fossor</i> (L.)	9 (0.8)	Predator
	<i>Elaphropus anceps</i> (LeConte)	1 (0.1)	Predator
	<i>Elaphropus</i> (Motschoulsky) sp.	2 (0.2)	Predator
	<i>Harpalus affinis</i> (Schränk)	158 (13.5)	Omnivore
	<i>Harpalus</i> Latreille sp.	6 (0.5)	Omnivore
	<i>Loricera pilicornis</i> (F.)	3 (0.3)	Predator
	<i>Poecilus lucublandus</i> (Say)	40 (3.4)	Predator
	<i>Pterostichus melanarius</i> (Illiger)	196 (16.8)	Predator
	<i>Stenolophus comma</i> (F.)	116 (9.9)	Omnivore
	<i>Stenolophus ochropezus</i> (Say)	1 (0.1)	Omnivore
	species 1	5 (0.4)	
	species 2	1 (0.1)	
Chrysomelidae	<i>Chaetocnema</i> Stephens sp. 1	6 (0.5)	Phytophage/Rhizophage
	<i>Chaetocnema</i> sp. 2	4 (0.3)	Phytophage/Rhizophage
	<i>Phyllotreta cruciferae</i> (Goeze)	147 (12.6)	Phytophage/Rhizophage
	<i>Phyllotreta striolata</i> F.	10 (0.8)	Phytophage/Rhizophage
Coccinellidae	<i>Coccinella septempunctata</i> (L.)	1 (0.1)	Predator
Cryptophagidae	<i>Atomaria</i> Stephens sp.	40 (3.4)	Mycetophage
Curculionidae	<i>Cleonis pigra</i> (Scopoli)	1 (0.1)	Phytophage/Rhizophage
	<i>Glocianus</i> Reitter sp.	1 (0.1)	Phytophage/Rhizophage
	<i>Sitona</i> Germar sp.	2 (0.2)	Phytophage/Rhizophage
	<i>Sphenophorus</i> Schoenherr sp.	24 (2.0)	Phytophage/Rhizophage
	species 1	2 (0.2)	
Dermestidae	<i>Attagenus</i> Latreille sp.	2 (0.2)	
	<i>Trogoderma</i> Dejean sp.	1 (0.1)	
Elateridae	<i>Aeolus mellillus</i> (Say)	21 (1.8)	Phytophage/Rhizophage
	<i>Hemicrepidius</i> Germar sp.	1 (0.1)	Phytophage/Rhizophage
	<i>Hypolithus</i> Eschscholtz sp.	1 (0.1)	Phytophage/Rhizophage
	species 1	1 (0.1)	
	species 2	1 (0.1)	
Nitidulidae	<i>Glischrochilus fasciatus</i> (Olivier)	2 (0.2)	Scavenger
	<i>Glischrochilus quadrisignatus</i> (Say)	80 (6.8)	Scavenger
	<i>Carpophilus</i> Stephens sp.	6 (0.5)	Scavenger
Scarabaeidae	<i>Aphodius</i> Hellwig sp.	1 (0.1)	Phytophage/Rhizophage

TABLE 1 continued...

Family	Genus/species	No. (%)	Functional Group
Staphylinidae	<i>Aleochara</i> Gravenhorst sp.	20 (1.7)	Predator
	<i>Anotylus insecatus</i> (Gravenhorst)	16 (1.4)	Scavenger
	<i>Anotylus rugosus</i> (F.)	5 (0.4)	Scavenger
	<i>Dinaraea angustala</i> (Gyllenhal)	30 (2.6)	Predator
	<i>Drusilla canaliculata</i> F.	32 (2.7)	Predator
	<i>Lathrobium</i> Gravenhorst sp. 1	1 (0.1)	Predator
	<i>Lathrobium</i> sp. 2	3 (0.3)	Predator
	<i>Philonthus</i> Stephens sp.	7 (0.6)	Predator
	<i>Quedius</i> Stephens sp.	2 (0.2)	Predator
	<i>Rugilus</i> Leach sp.	4 (0.3)	Predator
	<i>Scopaeus</i> Erichson sp.	4 (0.3)	Predator
	<i>Tachinus corticinus</i> Gravenhorst	16 (1.4)	Predator
	<i>Tachyporus nitidulus</i> (F.)	1 (0.1)	Mycetophage
	<i>Tachyporus</i> Gravenhorst sp.	1 (0.1)	Mycetophage
	Aleocharine sp. 1	4 (0.3)	Predator
	Aleocharine sp. 2	1 (0.1)	Predator
	Aleocharine sp. 3	1 (0.1)	Predator
	species 1	1 (0.1)	
TOTAL		1170	

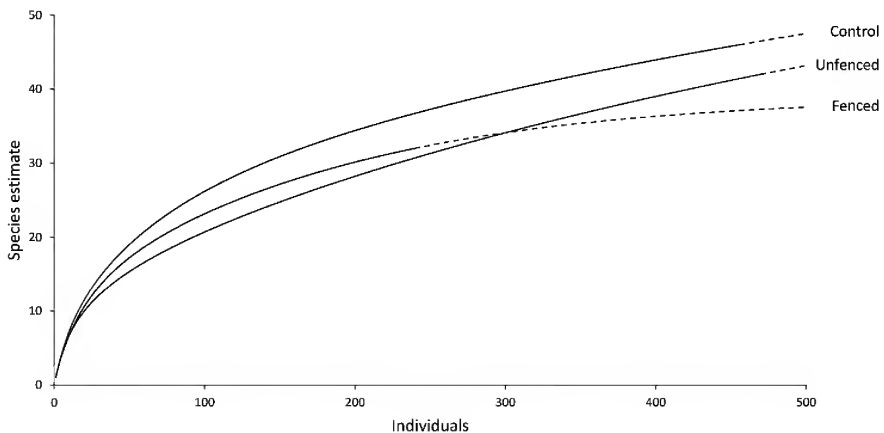


FIGURE 1. Rarefied estimates of beetle species richness in fenced or unfenced broccoli plots or control plots at Elora Research Station, early summer 2013. Rarefaction indicated with solid lines and extrapolation with dashed lines. Confidence intervals were removed for visual clarity.

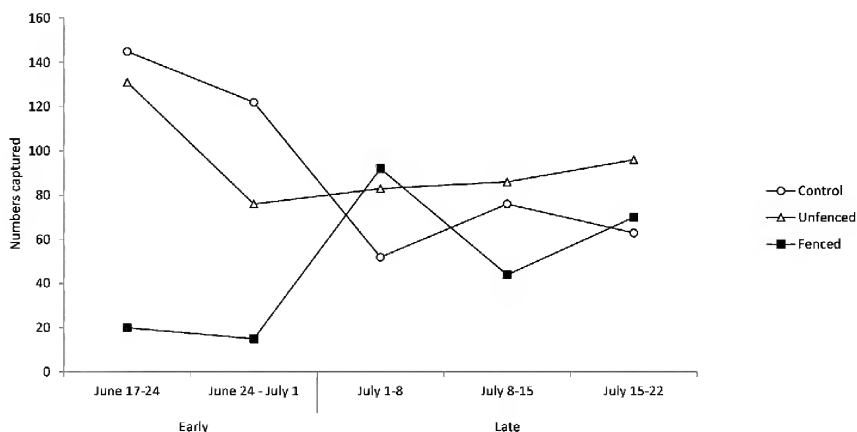


FIGURE 2. Total numbers of beetles captured in pitfall traps in fenced and unfenced broccoli plots and control plots at Elora Research Station, 2013.

early period, but differences among plot types were not recorded in the later period (Table 2, Fig. 3). Generic/species richness and diversity did not vary due to fencing (richness:  $F_{2,21} = 3.3$ ,  $P = 0.059$ ; diversity:  $F_{2,21} = 2.0$ ,  $P = 0.165$ ), period (richness:  $F_{1,3} = 0.1$ ,  $P = 0.803$ ; diversity:  $F_{1,3} = 0.05$ ,  $P = 0.837$ ), or the fencing  $\times$  period interaction (richness:  $F_{2,21} = 1.1$ ,  $P = 0.355$ ; diversity:  $F_{2,21} = 0.9$ ,  $P = 0.416$ ).

There were effects of fencing on most trophic groups of beetles (Table 2). Predatory beetle captures increased in fenced plots from the early to the late trapping periods, whereas omnivore captures remained lowest in fenced plots throughout the experiment (Fig. 3). Rhizophage and phytophage (i.e., potential pest) numbers decreased in control and unfenced plots from the early to the late trapping period, but remained low in fenced plots throughout the experiment (Fig. 3).

Ordination eigenvalues were 0.223 and 0.168 for the first two axes, respectively, and explained 47.7% of the cumulative variability in beetle species distribution. None of the frequently captured species were associated with fenced plots in four of the five sampling weeks (Fig. 4). All three plot types grouped closely for the sampling week of 1–8 July and were associated with *Bembidion quadrimaculatum* Say and a *Harpalus* sp. Three larger, common predatory species of Carabidae, *Harpalus affinis* (Schränk), *Poecilus lucublandus* (Say), and *Pterostichus melanarius* (Illiger), tended to associate with control plots during the later sampling weeks.

TABLE 2. Results of analysis of variance for effects of fencing (control, unfenced, fenced) and sampling period (early = 17 June – 1 July, late = 2–22 July) on numbers of all beetles and beetles categorized by trophic designation captured in broccoli plots at Elora Research Station, 2013.

Factors	All beetles		Predators		Omnivores	
	F	P	F	P	F	P
Fencing	6.2	0.008	1.2	0.312	6.7	0.006
Period	1.1	0.373	11.8	0.041	0.2	0.691
Fencing*period	5.8	0.010	3.8	0.039	1.3	0.289

Phytophages + Rhizophages	Scavengers <sup>1</sup>		Mycetophages	
	F	P	F	P
Fencing	14.2	< 0.0001	2.1	0.152
Period	12.7	0.038	0.1	0.773
Fencing*period	8.8	0.002	4.7	0.020

df: Fencing = 2, 21; Period = 1, 3; Fencing\*period = 2, 21

<sup>1</sup>Fencing\*period means not significantly different as indicated by Tukey’s HSD test,  $\alpha = 0.05$

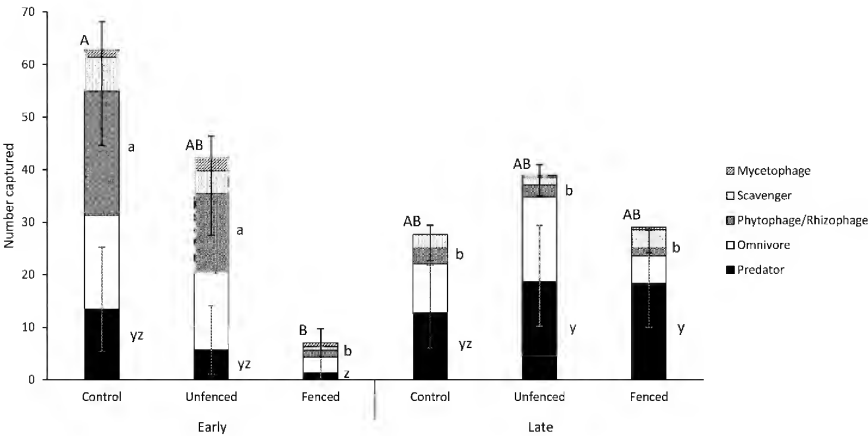


FIGURE 3. Mean (back-transformed) numbers of beetles categorized by trophic level captured per week in pitfall traps from broccoli plots that were unfenced or fenced or from control areas outside plots, at Elora Research Station, 2013. The experiment was divided into early (June 17–July 1) and late (July 1–22) trapping periods. For total beetles (A, B), omnivores (a, b) and predators (y, z) different letters indicate significantly different means using Tukey’s HSD test,  $\alpha = 0.05$ . Confidence intervals (95%) are shown only for predators and phytophages/rhizophages to improve visual clarity.



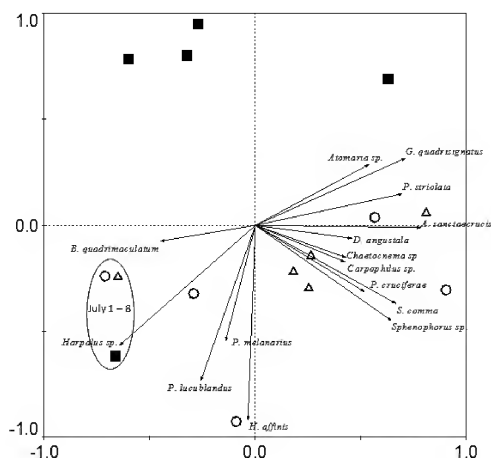


FIGURE 4. Principle component analysis biplot of beetle species and plot type for each sampling week in a broccoli field, at Elora Research Station, 2013. Species are shown by arrow vectors, empty circles are control plots, empty triangles are unfenced plots and filled squares are fenced plots. See Table 1 for full species names.

## Discussion

The community composition of beetles, particularly Carabidae, captured in this study were relatively similar to communities in other horticultural and field crops. *Harpalus*, *Pterostichus*, and *Anisodactylus* ground beetles were common in vegetable crops in western North Carolina, and, in addition, *Bembidion* spp. were common in eastern England *Brassica* crops (Hummel *et al.* 2002; Eyre *et al.* 2009). In Ontario sweet potato and carrot fields, the dominant ground beetle genera were *Pterostichus*, *Harpalus*, *Bembidion*, *Poecilus* and *Scarites*, and these as well as *Clivina*, *Agonum* and *Tachys* were common in Ontario field crops (maize, soybeans, wheat) (Belaoussoff *et al.* 2003; Brunke *et al.* 2009). While direct comparisons of beetle activity density or diversity between studies are difficult to make, similarities in predaceous and omnivorous ground beetle genera may mean community functionality – pest control and weed seed predation – is also relatively similar across crop types and/or locations.

Using fencing to exclude pests from broccoli had significant effects on beetle activity density, as fewer beetles were found in fenced compared to unfenced and control plots early in the study (17 June – 1 July). This result is due to the fact that most surface-active beetles were likely immigrating from field margins into the establishing crop at this time (i.e., Werling and Gratton 2008; Eyre *et al.* 2009), and fences effectively kept them from walking into plots. Despite influencing beetle abundance, fencing did not significantly impact generic/species richness or diversity. This result suggests that fencing does not

preferentially exclude particular taxonomic or functional groups of ground-dwelling beetles, but restricts access equally across beetle types.

We did not expect to capture many foliage-dwelling beetles in pitfall traps, but *Phyllotreta cruciferae* Goeze were abundant early in the study (138 early captures of 147 total captures) and comprised the majority (67%) of phytophages/rhizophages. This flea beetle is a widespread crucifer pest, causing defoliation and reduced yield (Tansey *et al.* 2008). Row cover can be used to protect young crucifer plants by excluding *P. cruciferae* (Andersen *et al.* 2006), but it also appears that exclusion fencing provides protection from this pest.

In the later part of the trapping season (2–22 July), beetle activity-density was not affected by fencing. Captures in control and unfenced plots tended to decrease compared to the early period, whereas captures in fenced plots tended to increase. This result suggests that beetles, of which predatory and omnivorous Carabidae and Staphylinidae were a large majority, moved into the fenced plots through gaps that occasionally opened at seams in fence panels or between the panels and the ground, or that species capable of flight entered over the top edge. Satiation can affect ground beetle movement, with hunger motivating dispersal (Fournier and Loreau 2001). High flea beetle levels outside fenced plots early in the season may have meant that predatory beetles frequently encountered prey (flea beetles or other prey) (Renkema *et al.* 2014), but as flea beetle numbers declined, predatory beetles may have increased their search activity and found their way into fenced plots.

No differences in beetle captures were found between unfenced and control plots, despite expecting greater beetle abundance and diversity in unfenced plots due to a barrier effect of adjacent fences. A tendency for association between control plots and some of the larger, more frequently captured ground beetle species (i.e., *H. affinis*, *P. lucublandus*, *Pt. melanarius*) later in the trapping period may be due to weedy vegetation around these plots or gaps in the fences or at lower edges that were not well maintained. In addition, these plots were less disturbed by other research activities associated with measuring fencing effects on pests, and these species may favour less disturbed habitats (Carmona and Landis 1999).

Using exclusion fencing to assist with broccoli pest management has the unintended consequence of excluding beneficial beetles from fields, or at least delaying their entry. Thus, biological control services could be reduced early in the season, and prevent natural enemies from limiting pest population growth (Hajek *et al.* 2007). In addition, effects of fencing small plots (this study) on beetle entry may be different than effects at a commercial scale, where a lower perimeter distance to fenced area ratio may further reduce likelihood of beetle entry. However, the fact that many beetles eventually permeated the fencing is promising for later season biological control services, but species-specific effects of predators on pests will need to be determined, as has been done in other southern Ontario horticultural crops (Brunke *et al.* 2009). The feeding ecology of rove beetles should be determined, particularly of species common in both this study and a previous survey of southern Ontario soybeans, such as *Drusilla canaliculata* and *Dinaraea angustata* (Brunke *et al.* 2014). Fencing designs that allow ingress (so that beetles can walk in but not out easily) and result in retaining abundant beetle populations in fenced fields, leading to greater insect pest predation and agroecosystem services, should also be investigated.

## Acknowledgements

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**POSTER AND PRESENTATION ABSTRACTS  
ENTOMOLOGICAL SOCIETY OF ONTARIO  
ANNUAL GENERAL MEETING**

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**Poster abstracts**

**Sublethal impacts of pesticide exposure on bumble bees and squash bees, and a wild pollinator monitoring programme for Ontario**

Agar, E., G.L. Baron, M.J.F. Brown, D.S. Chan, R.J. Gill, V.A.A. Jansen, A. Pindar, C. Rubens, D.A. Stanley, and N.E. Raine

**New range records from a headwater in a beef pasture provide evidence of agricultural streams as important habitat for invertebrates**

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Headwater streams contribute to the biodiversity of freshwater systems and provide important habitats for invertebrates in agricultural landscapes. Compared to higher order and larger streams, little is known about the ecology of small headwater streams and the taxa associated with them. We report on 28 invertebrate species found in a small headwater stream on a beef pasture in central Ontario (Canada) over two years. Two species, *Lepidostoma liba* (Ross) (Trichoptera: Lepidostomatidae) and *Pericoma albitarsis* (Banks) (Diptera: Psychodidae) are first provincial records for Ontario, and several others are significant range extensions or gap infills. We discuss the importance of continued sampling in small, overlooked habitats to better understand species diversity.

**Where do the army worm come from? Using stable isotopes to study the migration of *Pseudaletia unipuncta***

Doward, K., J.N. McNeil, and K.A. Hobson

In Canada, many lepidopteran pest species, such as the true armyworm (*Pseudaletia unipuncta*), are seasonal migrants. Consequently, as little is known about their origin other than “they come from the south”, current management strategies are generally based on pheromone trap catches to estimate the density of immigrants. However, if immigrant populations come from specific areas year after year, one could potentially estimate the size of immigrant populations based on overwintering conditions at the source site.

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The existence of a latitudinal continental spatial hydrologic scale for deuterium in precipitation (and thus in the plants growing at different sites) provides a means of determining the natal origin of migrant species. Consequently, I am using hydrogen isotope ratios ( $\delta^2\text{H}$ ) to determine the degree of intra- and inter-year variability in armyworm moths captured in London, ON. Initially, I reared cohorts of larvae on vegetation (*Hordeum vulgare*) treated with water having different concentrations of deuterium to establish the relationship between water  $\delta^2\text{H}$  and wing chitin  $\delta^2\text{H}$ . As there are three periods of armyworm flight activity (immigrants, residents and emigrants) I am now analysing the wings of field-collected moths that were captured in different years and at different times during the seasons.

Our working hypothesis is that within a year, immigrants captured in spring will have significantly different  $\delta^2\text{H}$  profiles than those in summer and fall, as they will have fed on host plants growing in locations much further south while residents and emigrant populations will have fed on local vegetation. The inter-year comparison will allow me to determine to what extent the origin of immigrants varies from one year to the next.

### **Range expansion pattern of *Carabus granulatus* Linnaeus (Coleoptera: Carabidae) in eastern North America**

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*Carabus granulatus* Linnaeus (family: Carabidae) is native to Europe. It was first introduced to North America in 1890 in St. John, New Brunswick. In 2011, *C. granulatus* was collected in Moosonee, Ontario, extending the known distribution northward by 200 kilometers. Typically, invasive species follow one of three different types of radial expansion curves, based on the dispersal characteristics (Shigesada and Kawasaki 1997). Using published records of previous sightings, we analyzed the expansion rate of *C. granulatus* in eastern North America. *C. granulatus* likely dispersed by diffusion, (Type 1 expansion curve) through eastern North America, characteristic of other coleopteran invaders.

### **Urban effects on blow fly species diversity across Canada**

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We tested whether the ecozone an urban area is located in, or the urban area itself had a greater effect on forensically relevant blow fly (Diptera: Calliphoridae) species composition across Canada. We mailed bottle traps to 32 collaborating OPP and RCMP locations across Canada in 2011, 2012 and 2013. We found that blow fly species composition in urban areas across Canada were similar over a range of ecozones.



### **Biosurveillance of Alien Forest Enemies (bioSAFE): Pathway analysis and diagnostics of Asian Longhorned Beetle**

Roe, A.D., C. Duff, R. Hamelin, K. Hoover, M. Keena, C. Landry, M. Javal, S. Juan, A. Roques, and Y. Wu

### **Efficacy of insecticides for control of brown marmorated stink bug (*Halyomorpha halys* Stål) nymphs in Ontario**

Scott-Dupree, C.<sup>1</sup>, A. Gradish<sup>1</sup>, K. Hunter<sup>1</sup>, H. Fraser<sup>2</sup>, and T. Garipey<sup>3</sup>

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*Halyomorpha halys*, the brown marmorated stink bug (BMSB), is an invasive agricultural and household pest native to East Asia. Identified as established in Ontario in 2012, its range is expanding into agricultural areas. Sustainable pest management options must be identified to prevent significant economic loss for Ontario growers. Older, broad-spectrum insecticides appear to be most effective for managing BMSB but may be undesirable due to non-target impacts. Therefore, screening of alternative insecticides are required. Results of laboratory studies using direct contact toxicity tests of novel insecticides (alone and in combination) on fifth instar nymphs are discussed.

### **Japanese knotweed Psyllid *Aphalara itadori*: A biological control agent for invasive knotweeds**

Skuse, T

The psyllid *Aphalara itadori* has recently been approved for open release to help control invasive knotweed plants (*Fallopia* sp.) in Canada. Before widespread releases into knotweed-infested areas can occur, there is a need to further investigate how these insects will establish in novel ecosystems. We will present data from ongoing research partnered with Agriculture and Agri-food Canada about the ecology of a new bug entomologists can expect to see more of in the near future.

### **Overwintering energetics and microhabitat selection in the western bean cutworm**

Turnbull, K.F., J.N. McNeil, and B.J. Sinclair

Overwintering insects risk depleting energy stores at increased temperatures due to an elevation in metabolic rate. However, insects can conserve energy behaviourally by selecting a microhabitat that buffers against higher temperatures, or physiologically through metabolic plasticity. The western bean cutworm (*Striacosta albicosta*) appears to use both strategies; the soil depth of prepupal chambers is a determinant of overwintering temperatures, and warmer conditions induce a decline in the thermal sensitivity of metabolism. We demonstrate that soil texture and temperature at the time of burrowing influence overwintering site selection, which may have implications for overwintering success.

## Distribution of Syrphidae in northern Ontario

Vezensyi, K.<sup>1</sup>, J.H. Skevington<sup>2</sup>, W.J. Crins<sup>3</sup>, J. Schaefer<sup>1</sup>, D. Beresford<sup>4</sup>

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I report on the distribution of an important pollinator group, hover flies (Syrphidae), from Ontario's northern boreal region, and Akimiski Island, Nunavut. Little is known regarding the distribution of this family in the more remote parts of northern Ontario. To date, I have identified 49 species from 251 individuals, with about 400 still to be processed. Fifteen of these are range extensions, and provide new records of rare species. This project will add to our understanding of the distribution of this important group.

## Presentation abstracts

### Intra-caste variation in a eusocial sweat bee, *Lasioglossum laevisissimum*

Awde, D

Sweat bee studies typically explore how abiotic and environmental factors influence female social status but rarely focus on intra-caste variation. In a southern Ontario *Lasioglossum laevisissimum* population, spring and summer queens were similar in size and ovarian development but differed in foraging behaviour. Half of workers had some ovarian development and 20% of workers had substantial ovarian development, suggestive of queen-like behaviour. These queen-like workers were similar in size to classic workers. Our results show considerable intra-caste variation, emphasizing the behavioural flexibility inherent in *L. laevisissimum* castes.

### Is competition superior to parasitism for biological control? The case of spotted wing drosophila (*Drosophila suzukii*), *Drosophila melanogaster* and *Pachycrepoideus vindemmiae*

Dancu, T.<sup>1</sup>, T.L.M. Stemberger, P. Clarke, and D.R. Gillespie

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*Drosophila suzukii* and *Drosophila melanogaster* coexist with overlapping resource use. When sharing resources in the lab, *D. melanogaster* outcompetes *D. suzukii*. We allowed adult *D. suzukii* and *D. melanogaster* females to compete for access to a common oviposition resource in dyadic and population scale experiments and a closed field simulation cage experiment with a generalist *Drosophila* parasitoid, *Pachycrepoideus vindemmiae*. Competitor identity was found to significantly affect *D. suzukii* numbers over the presence of *P. vindemmiae*. Competition by *D. melanogaster* appears to be more effective as biological control for *D. suzukii* than a parasitoid in this system. <http://dx.doi.org/10.1080/09583157.2016.1241982>

## **Effects of cold acclimation on structure and transport function in insect ionoregulatory tissues**

Des Marteaux, L.<sup>1</sup>, and B.J. Sinclair<sup>1</sup>

*1. University of Western Ontario*

Chill-susceptible insects lose ion and water homeostasis and develop chilling injuries when exposed to cold, but prior cold acclimation allows insects to maintain homeostasis to lower temperatures and avoid injury. The mechanisms underlying cold acclimation are not well-understood, but likely involve modification of cell/tissue structure and/or epithelial transport function. The insect hindgut and Malpighian tubules (major sites of ionoregulation) are likely targets for these modifications. In adult *Gryllus pennsylvanicus* crickets we characterized the effects of cold acclimation on structural and ionoregulatory gene expression, hindgut cytoskeletal stability, and Malpighian tubule secretion. We found that cold acclimation alters both active transport function and cell structure, and discuss how these modifications may prevent chilling injury and loss of homeostasis.

## **Staphylinid diversity across a Neotropical elevation gradient**

Dolson S., E. Loewen<sup>1</sup>, K. Jones<sup>1</sup>, and M.A Smith<sup>1</sup>

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Climate change is altering the diversity, abundance, and distribution of natural populations. Of these populations, those in tropical montane areas are expected to be the first affected by changing conditions. Studying the effects of abiotic and biotic conditions on tropical community composition and distribution can provide insight into what community changes may occur in the future as these factors shift. Elevation gradients provide a useful model system to study these effects since many conditions covary as elevation increases, namely temperature (decreasing) and precipitation (increasing). Understanding community response to changing conditions is particularly important in arthropods as most are understudied despite their abundance and importance in various ecological populations. Therefore, I focus my research on defining the staphylinid communities across an elevation gradient in the Area de Conservación Guanacaste in Costa Rica and determining how they are affected by the changing environmental factors. Continuous collections have been made at 8 sites across a 1500 m elevation gradient since 2008 and to quantify diversity we will use DNA barcodes and classic taxonomy when available. Investigation into these communities will not only provide insight into important yet unknown biogeographical patterns of an understudied taxa but also into how ecological communities may respond to changing climatic variables.

## **Evidence for extended parental care in the eastern carpenter bee, *Xylocopa virginica***

Duff, L

The eastern carpenter bee, *Xylocopa virginica* has two provisioning flight periods, one to provision nest-mates from late April to early May and another to provision brood cells from late May to early July. In St. Catharines, Ontario we observed both female and

male bees leaving nests in late August and in the beginning of September. During this time, we made novel observations that worn and unworn females made pollen provisioning trips to nests. In Ontario, carpenter bees have little time to provision a second brood, so late summer provisioning flights may be intended as extended parental care.

### **New pest issues in Ontario horticulture in 2016**

Fraser, H

*Ontario Ministry of Agriculture, Food and Rural Affairs*

Invasive insects have resulted in the disruption of many well-established integrated pest management programs. In 2016, the European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), was detected at several locations in southern Ontario. The find represents the first confirmation of this pest in North America. The presence of pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), not though to overwinter successfully under typical Ontario winters, has resulted in crop loss in both greenhouse and field peppers. Pepper weevil has the potential to become a community-level pest under certain conditions. Both pests are discussed in the context of Ontario production.

### **Status of ambrosia beetles in high-density apple orchards in southern Ontario**

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*1. Ontario Ministry of Agriculture, Food and Rural Affairs*

Wood-boring ambrosia beetles have been reported as an emerging issue in the United States where they have caused substantial damage in high density apple orchards over the last few years. A preliminary survey was conducted to determine the range, abundance and diversity of ambrosia beetles in 13 high density apple orchards located in southern Ontario. A survey for ambrosia beetles was conducted in 13 high density apple orchards. Six species of ambrosia beetle were identified including *Anisandrus dispar*, *A. sayi*, *X. germanus*, *Xyleborus obesus*, *Monarthrum mali*, and *Xyleborinus saxesenii*. Beetle numbers in 2016 were low relative to those reported in other jurisdictions in 2015.

### **Discovery of a parasitoid attacking the invasive swede midge in Ontario**

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The invasive alien swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae), was first recorded from canola fields in Shelburne, ON in 2003. In 2016, weekly plant collections were made at four canola fields in this area in order to look for the presence of swede midge parasitoids. A platygastriid was found in all fields over a 6-week period, with parasitism rates ranging from 4% to 20%. Future studies are planned to learn more about this parasitoid and its biological control potential against the swede midge.

***Trichoplusia ni* (Hübner) attraction to transgenic *Solanum lycopersicum* (L): Exploring transgenic trap crops for the cabbage looper moth**

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New pest management strategies are required to counter insecticide-resistance in populations of greenhouse insects, for example *Trichoplusia ni* (Hübner), the cabbage looper moth. One alternative is to use trap crops - planting attractive, disposable plants within the main crop arrangement to draw pests away from the crop. In this study, transgenic *Solanum lycopersicum* (L) tomato was genetically modified to over-express the gene that regulates carotenoid cleavage dioxygenase (CCD) enzymes, altering production of tomato volatiles. The objectives of the study are to verify and assess cabbage looper moth olfactory attraction to volatiles emitted by transgenic tomato lines relative to wild-type.

**An investigation of Staphylinidae functional diversity along a neotropical elevation**

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Staphylinidae is a hyperdiverse and abundant family of Coleoptera found worldwide. My research focuses on how Staphylinidae functional diversity varies with environmental changes associated with the elevation gradient (e.g. temperature decreases with increasing elevation) on Volcán Cacao in the Área de Conservación Guanacaste (ACG) in northwestern Costa Rica. Since 2008, multiple samples have been taken at various elevations (10m above sea level – 1500m above sea level) and in the three distinct forest types (dry forest, rainforest and tropical montane cloud forest (TMCF)) that characterize Volcán Cacao. Through morphometric measurements and discrete variables such as exoskeleton texture and colour, I will investigate the changes in Staphylinidae functional morphospace along the elevation gradient and between the forest types. Like many tropical arthropods, our understanding of staphylinids suffers severe taxonomic impediments due to the relative scarcity of taxonomists working on them, many unnamed and undescribed species, and the many described species that may represent multiple separate species. My proposed research will add to our limited knowledge on neotropical staphylinids and test predictions regarding how abiotic factors such as temperature and precipitation affect functional measures of diversity.

**Alien (spider) invaders: *Enoplognatha ovata* wreak havoc on a coastal arthropod community**

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*Enoplognatha ovata* (the candy stripe spider) is a theridiid introduced to North America from Europe, and common on the West Coast. Much-studied because of its striking colour polymorphism, its ecology has thus far attracted little interest. Here we report on the

remarkable predation behaviour of this unassuming spider, observed during a longitudinal study of a field site on Vancouver Island, BC. We show how *E. ovata* uses theft, piracy, and strategic web-building to carve a trail of havoc across the sand dunes, with the potential for significant impact on the native arthropod community, including hymenopterans and other spiders.

### **Wing interference patterns (WIPs) – a potential tool for the identification of Sciomyzidae (Diptera) of eastern Canada and the adjacent U.S. states**

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Wing interference patterns (WIPs) are specific colour patterns developed through thin film interference that appear on translucent insect wings, and are a potential source of useful taxonomic data. WIPs were discovered in 88 species across 21 genera of Sciomyzidae (Diptera) from eastern Canada and adjacent U.S. states. Moderate to high interspecific variation and low to moderate intraspecific variation of WIPs was discovered, along with one record of sexual dimorphic WIPs. WIPs of Sciomyzidae are of most value in a taxonomic identification key as a supplemental taxonomic trait to improve difficult couplets, such as those that require examination of internal genitalia.

### **The value of entomology field courses**

Otis, G.W

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Steve Marshall and I began teaching Field Entomology 20 years ago. Many undergraduate students get little exposure to field activities. Providing them those opportunities can be transformative. Our course has turned-on many students to the world of insects and field research, with many now contributing professionally to the discipline of entomology. Major ingredients for a successful course are keeping students warm, clean, well-fed, and happy. The sites where the course is taught must be interesting biologically and hopefully physically appealing. We encourage live-in courses at field stations, not weekday-daytime camps, where students become absorbed in course activities.

### **Effect of gut-associated yeasts on *Drosophila melanogaster* performance**

Jiménez Padilla, Y.<sup>1</sup>, M-A Lachance<sup>1</sup>, and B.J. Sinclair<sup>1</sup>

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Yeasts are an important part of the *Drosophila* diet due to their nutritional value, and as such, yeasts have the potential to affect phenotype. Furthermore, living yeast might also interact with the fly as its host in ways we have not yet discovered. While yeasts are an integral part of the fly gut community, most studies have focused on bacteria alone. However, *Lachancea khyveri*, a yeast commonly associated with *Drosophila* in nature,

affects fly physiology, and the magnitude of the effect is dependent on the yeast being alive or dead.

### **Insecticide impacts on bumblebees: from colony founding to pollination services**

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Recent concern over global pollinator declines has led to considerable research on pesticide impacts. Here we report results from a series of studies examining to what extent field-realistic insecticide exposure can lead to significant sublethal impacts on individual behaviour (e.g. reduced queen colony founding success and impaired worker learning and foraging), colony function (e.g. effects on growth rates and forager recruitment), and the critical ecosystem services bumblebees provide to crops and wild plants. Taken together these effects could have widespread implications for the stability of wild pollinator populations, sustainable production of pollinator limited crops, and maintaining wild plant biodiversity.

### **Experimentally induced alloparental care in a small carpenter bee**

Richards, M. and V. Lewis.

Alloparenting, in which adults help to raise non-descendant offspring, is the hallmark of both cooperatively breeding and eusocial animal groups. In the small carpenter bee, *Ceratina calcarata*, mothers sometimes produce very small daughters, reminiscent of eusocial workers, suggesting retention of a complex social trait, alloparenting, in this secondarily solitary bee species. Experimental removal of mothers does induce the smallest daughters to feed their siblings, but natural orphaning always results in brood predation. Thus the necessity for maternal care precludes the possibility of alloparenting and explain why this bee has reverted to solitary behaviour.

### **The mating dynamics of western black widows: new insights from field observations**

Scott, C.<sup>1</sup>, S. McCann, and M.C.B. Andrade

1. *Department of Biological Sciences, University of Toronto Scarborough; Department of Ecology and Evolutionary Biology, University of Toronto*.

The western black widow, *Latrodectus hesperus*, has been the subject of several experimental studies of sexual behaviour and communication, yet our knowledge of its natural history remains incomplete. We conducted a six-month longitudinal study of a dense population of *L. hesperus* on Vancouver Island, BC. We report new information about phenology, and how this intersects with changing modes of sexual selection, including the timing of mate-searching and mating, the frequency of mate-guarding, and the intensity of male-male competition. These data are essential for designing new behavioural experiments and interpreting results of earlier studies.

### **Evaluating the interaction between buckwheat *Fagopyrum esculentum* (Polygonaceae) and wireworm (Coleoptera: Elateridae) species**

Scott, I.M.<sup>1</sup>, Y.L. Bohorquez Ruis<sup>1,2</sup> and J.N. McNeil<sup>2</sup>

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Click beetle larvae or wireworms are considered pests due to the damage caused by feeding on the root systems of agricultural crops. Field studies in Atlantic Canada using buckwheat in rotation with potato resulted in a reduction of *Agriotes* spp. wireworm populations. Microplot trials with buckwheat and an Ontario species, *Limonijs agonus* (Say), demonstrated a negative effect on larvae during a 3 week interaction. Laboratory soil olfactometer experiments found no evidence that *Agriotes sputator* L. were deterred by buckwheat at germinating, branching and flowering stages relative to red spring wheat (*Triticum* spp.) and island barley (*Hordeum vulgare*). In the greenhouse, a 3 week, no choice feeding assay determined no difference in weight and mortality of *A. sputator* larvae when fed buckwheat or barley. However, in contrast to barley, the wireworm herbivory did not affect buckwheat growth, suggesting that buckwheat roots may produce anti-feedants.

### **Comparison of complexity and intelligence among cynipid gall wasps and wild roses**

Shorthouse, J

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One approach to understanding the life history strategies of phytophagous insects and their host plants is to compare their relative complexity and even their intelligence. Although all zoologists are comfortable discussing the relative complexity of their study animals, most are likely uneasy when considering insects and plants as intelligent. Here I use gall wasps of the genus *Diplolepis* (Family Cynipidae) and their host shrub roses of the genus *Rosa* (Family Rosaceae), to question which is more biologically complex and intelligent, gall wasps represented by both larvae and adults, or their host shrubs?

Because adult cynipids have a nervous system and fly to locate oviposition sites, whereas shrubs are sessile and without a nervous system, most biologists likely agree that cynipids are more complex than plants. Cynipid larvae are also considered complex because they control plant growth causing their larvae to be encased by protective layers of high quality plant food in new organ-like structures. However, cynipids spend most of their lives as either larvae or prepupae and only two weeks as pupae and adults. Immature stages are immobile and have no opportunity to make decisions.

Wild roses with their modular structures and clonal growth forms are also complex organisms as they search for optimum habitats, assess signals and make decisions as they slowly grow the shoots and roots of their ramets. Roses have growing points both above and below the ground where they receive and assess information on light, temperature, touch, soil structure, insect attack, microbes, gravity, gradients of water, nutrients, minerals and toxins, and chemical signals from other plants.

Rose shrubs respond to more signals than cynipids suggesting a transduction



network of substantial complexity. Even though the leaves, buds, flowers, stems, and roots of a rose bush operate with some degree of independence, they are integrated to form a complex organism and they accomplish this without overall controlling organs or a nervous system. They plan their growth trajectory and there is evidence that each perceived signal presents different problems that requires intelligent mastery.

A common definition of intelligence in plants includes an intrinsic ability to process information from both abiotic and biotic stimuli that allows optimal decisions about future activities in a given environment in order to solve problems. Roots of plants, for example, investigate, search, survey, examine, and discover.

While we debate the legitimacy of using terms such as learning, memory, decision-making and intelligence when studying plants, and the ways in which they react to insect attack, it is becoming easier to accept that some plants exhibit brainy behaviour in the absence of brains. There is even evidence that some plants can become primed after repetitive insults of heat, herbivores, drought, cold, etc. such that they respond more quickly in the future to these conditions. In other words, they exhibit memory.

If intelligence reflects the capacity to solve problems, then a case can be made that shrub roses are more intelligent than cynipids because they react to a wider range of problems both above and below the soil surface. It appears that wild roses have become information processing entities of such complexity, integration, and adaptive competence that they rival those of many advanced animals and current electronic systems. We are reminded that after Charles Darwin studied the sensory capabilities of plant roots, he asked us to think of plants as a kind of up-side down animal with the main sensory organs and ‘brain’ on the bottom deep underground and its sexual organs on top.

### **Assessment of the role of neonicotinoid seed treatments to manage early season corn pests**

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A four year study is underway in Ontario, Canada to evaluate the utility of neonicotinoid insecticide seed treatments in a corn and soybean production system. The objectives of this study are to 1) determine the key early season insect pests and their distribution in Ontario corn and soybeans, 2) develop early season insect pest risk assessments tools, and 3) to measure the economic impact of neonicotinoid seed treatments for early season pest control in these crops. Approximately 150 replicated strip plots were conducted in 2014 and 2015 on cooperator’s farms comparing corn and soybeans treated with a fungicide or fungicide + neonicotinoid seed treatment. Early season foliar and soil insect pest presence were assessed using destructive sampling and bait trapping methods to determine species composition and damage levels. Plant populations were measured, plots were harvested by the cooperators and yield data were reported to the researchers. The key wireworm and grub species found in Ontario corn and soybeans will be reported along with the results of wireworm bait trap method comparisons. Early indications of monitoring for soil pests in the fall or spring suggest that applying insecticides based on the observed presence of the pest will be challenging. The results of this study will also provide context

to the overall discussion of the value of neonicotinoid seed treatments in corn and soybeans in Ontario, Canada.

### **When ants get up; they sure get brown**

Smith, M.A

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Using a long-term collection of ants from an neotropical elevation gradient in Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica we have tested the prediction that in cool environments ectotherms should be, darker to maximize heat gain and larger to minimize heat loss. We found that assemblages from higher elevation sites (cloud forests) were darker than rain or dry forests. Mid-elevation sites were characterised by the greatest range of lightness values. Furthermore, we found that cloud forest ant assemblages are becoming significantly lighter with time, suggesting the arrival of downslope assemblage members coincident with the increasing dryness and brightness that is affecting contemporary cloud forests.

### **Evaluating the efficacy of pure and hybrid populations of a biological control agent**

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The success of suppressing densities of invasive plants by re-introducing natural enemies from the weed's native range may be quite variable across the invaded range. One important source of variation may be the biocontrol agents themselves. Often they originate from multiple locations, and it is usually unknown which introduction led to establishment or whether agents from different locations have hybridized. Hybridization between genetically distinct populations can be beneficial (e.g. hybrid vigour) or detrimental (e.g. outbreeding depression) to biological control, and can drive rapid eco-evolutionary dynamics. We genotyped 15 ragwort flea beetle (*Longitarsus jacobaeae*) populations in northwestern Montana, USA where beetles originating from Italy and from Switzerland were released to control the noxious weed, tansy ragwort (*Jacobaea vulgaris*). We found that almost half of the populations contained hybrid individuals, while the rest were of Swiss origin and none of pure Italian origin. We then tagged 60 tansy ragwort rosettes at each of 9 sites where sufficient numbers of plants were available: 6 with Swiss beetles only and 3 containing hybrids. To assess the efficacy of biocontrol we applied insecticide to half of the tagged plants (the controls) to exclude beetles, and we monitored the plants for 2 years. Plants that were exposed to ambient levels of feeding by the flea beetles had 52% higher mortality, 17% lower flowering success, and those that flowered had 59% lower seed production than low herbivory plants. At hybrid sites plant mortality tended to be higher, and flowering success

and seed production lower, although only plant fecundity differed significantly from sites with Swiss beetles. Our results indicate that Swiss beetles provide successful control of tansy ragwort at high elevation sites in Montana, where pure Italian beetles had difficulties establishing due to a mismatch with the climate. Moreover, hybridization between Swiss and Italian beetles may have benefited biocontrol, since hybrids appear more effective than Swiss beetles. These findings reveal the power of biological control, and highlight the importance of hybridization in shaping the outcome of biocontrol programs, and suggest that taking an evolutionary perspective in implementing biocontrol could increase success rates.

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